

## Making of Chemically Competent Cells with $\text{CaCl}_2$

Before Starting:

- Grow culture overnight at  $37^\circ\text{C}$  and 225 rpm
  - Prepare stock of  $\text{CaCl}_2$  0.5M and  $\text{CaCl}_2$  1M and sterilize
1. Place  $\text{CaCl}_2$  0.5 M and  $\text{CaCl}_2$  1M containers on ice
  2. Preheat 40 mL of LB broth on a Corning tube of 50mL in water bath at  $37^\circ\text{C}$  for approx. 15 min
  3. Add 1.5mL of the overnight culture to the 40 mL of LB broth
  4. Place on shaker at  $37^\circ\text{C}$  and 225 rpm until the  $\text{OD}_{600}$  is between 0.4 - 0.6
  5. Place on ice for 20 min
  6. Centrifuge at 3000 rpm and  $4^\circ\text{C}$  for 15 min
  7. Discard supernatant. Add 20 mL of precooled  $\text{CaCl}_2$  0.5M and very carefully resuspend with micropipet
  8. Incubate on ice for 20 min
  9. Centrifuge at 3000 rpm and  $4^\circ\text{C}$  for 15 min
  10. Discard supernatant. Add 4 mL of precooled  $\text{CaCl}_2$  1M and very carefully resuspend with micropipet
  11. Incubate at  $4^\circ\text{C}$  all night for use the next day.